STAIN TECHNOLOGY

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ON THE STRUCTURE AND STAINING OF STARCH GRAINS OF THE POTATO TUBER[1]

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ABSTRACT.—**Procedures are described for the differential staining of starch grains of the potato tuber with hematoxylin, and for double staining with safranin 0 and fast green FCF. The staining effects obtained are made possible by the action of a swelling agent. Staining with hematoxylin is preceded by the swelling action of formaldehyde. In staining with safranin 0 and fast green FCF, the formaldehyde is added to the staining solution. The results obtained are as follows: (1) a clavate-shaped, central structure composed of small particles arranged in definite layers is revealed within the grain; (2) differential staining of the locus of the grain and the lamellae alternating with it in a small region around the longitudinal axis of the grain; (3) the simultaneous staining and separation of the grain into a cone-shaped peripheral portion and a spherical body containing the locus of the grain which emerges from it; and (4) differential staining of a ring or layer of substance around a spherical refractive body within the grain.**

When starch grains from the potato tuber are mounted in water and examined under the microscope, one can observe the locus of the grain around which numerous eccentric layers of starch are formed (Fig. 1).

Some selective staining of the locus of the grain and alternate lamellae may be obtained by the following procedure:

1. Smear commercial potato starch on a slide previously treated with Szombathy's[2] gelatin adhesive, as modified by Haupt[3] and Adams[4].

[1]Contribution No. 423 from the Department of Botany and Plant Pathology, Kansas State College of Agriculture and Applied Science.

[2]Szombathy, K. 1918. Neue Methode zum Aufklefen von Paraffinschnitten. Zts. Wis. Mikr., **34**, 334–6.

[3]Haupt, A. W. 1930. A gelatin fixative for paraffin sections. Stain Techn., **5**, 97–8.

[4]Adams, J. E. 1940. A procedure for staining filamentous algae and fungi on the slide. Stain Techn., **15**, 15.

STARCH GRAINS OF THE POTATO TUBER

FIG. 1. Eccentric striations of the grain.

FIGS. 2 and 19. Differential staining of the locus and alternate lamellae in a small region around the longitudinal axis of the grain.

FIG. 3. Clavate-shaped central structure unstained or slightly stained with hematoxylin.

FIG. 4. Clavate-shaped central structure showing numerous tiny granules which stain blue with hematoxylin.

FIGS. 5 and 20. Clavate-shaped central structure showing numerous brown-black appearing granules arranged in definite layers after staining with hematoxylin.

FIG. 6. Clavate-shaped central structure showing arrangement of needle-like crystals.

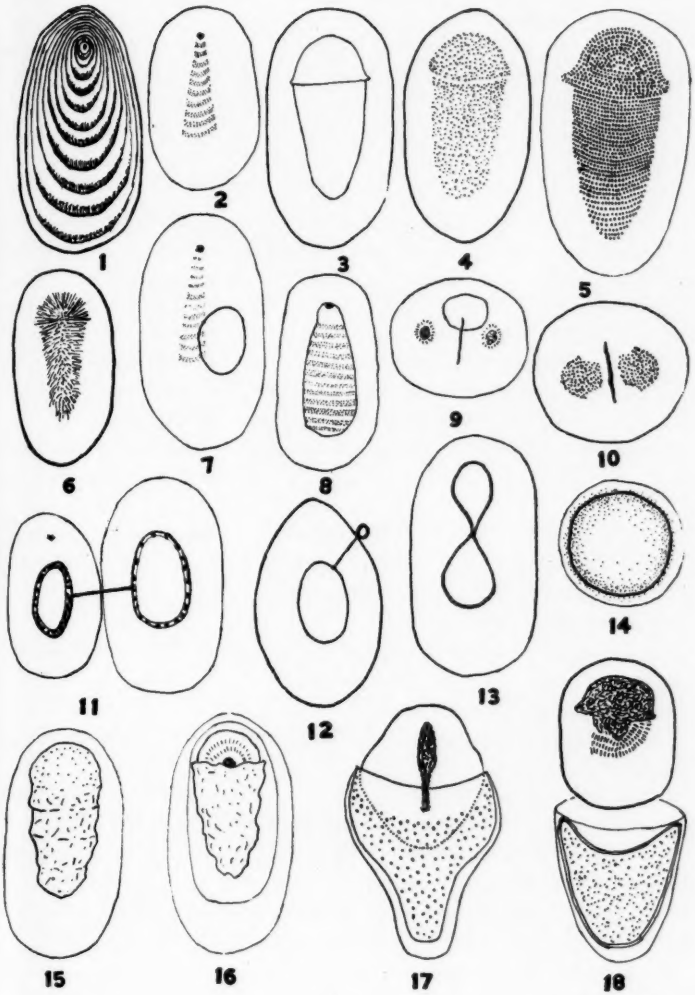
FIGS. 7, 8, and 9. Showing location of spherical refractive body within the starch grain.

FIG. 10. Showing clavate-shaped central structure in a compound grain.

FIG. 11. Reticulate ring of substance around the spherical refractive body which stains deep blue with hematoxylin.

FIGS. 12, 13, and 14. Showing layer of substance around the spherical refractive body which stains green with fast green FCF while the remainder of the grain stains red with safranin O. On standing the green color spreads until the red is completely obscured, Fig. 14.

FIGS. 15–18. Showing differential staining and stages in the separation of the grain into two parts.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2. Treat 10 minutes with a 3% solution of commercial formaldehyde.

3. Expose 3 minutes to the fumes of commercial formaldehyde in a closed container.

4. Stain 30 minutes in the following solution of hematoxylin: 95% alcohol, 79 cc.; distilled water, 10 cc.; a 2% solution of hematoxylin (certification No. FH-10 used by author) in absolute alcohol, 5 cc.; a 2% aqueous solution of aqueous ferric chloride, 5 cc.; normal HCl, 1.3 cc.

5. Rinse in tap water, 10 minutes.

6. Dehydrate with alcohol. Clear in xylene. Mount in balsam.

In these preparations the locus of the grain and alternate lamellae in a small region around the longitudinal axis of the grain become rather conspicuous (Figs. 2 and 19). If the time of exposure to the fumes of commercial formaldehyde is increased from 3 to 30 minutes, the small particles composing the locus and alternate lamellae form needle-shaped crystals arranged at right angles to the lamellae (Fig. 6).

Considerable optical differentiation of the central and outer portions of the potato starch grain may be obtained by the following procedure:

1. Fix small pieces of recently dug potato tubers in CRAF killing fluid (1% chromic acid, 50 cc.; 10% acetic acid, 35 cc.; formaldehyde, 37–40% aqueous, 15 cc.), 24 to 48 hours.

2. Wash in running water.

3. Treat for 12 hours in each of the following: dioxan and water, 1:2; dioxan and water, 2:1; and 3 changes of pure dioxan.

4. Infiltrate with paraffin.

5. Section and flatten the paraffin ribbon by floating it on warm water at 35–40° C.

6. Mount the paraffin sections on a slide previously treated with Szombathy's gelatin adhesive, as modified by Haupt (*loc. cit.*).

7. Expose the sections to the fumes of commercial formaldehyde in a closed container, 36 hours.

8. Remove slides from the closed container and permit them to dry.

9. Dissolve off the paraffin with xylene, pass into 95% alcohol, then stain in the hematoxylin solution described above, 12–24 hours.

10. Rinse in tap water, dehydrate with alcohol, clear in xylene and mount in balsam.

The starch grain should show an inner clavate structure, dome-shaped at locus end, which remains unstained or only lightly stained

while the surrounding portion of the grain stains a light blue. (Fig. 3.) If the time of exposure to the fumes of formaldehyde is increased, numerous blue-staining granules will appear in the clavate structure (Fig. 4). If the time of exposure to the fumes of formalde-

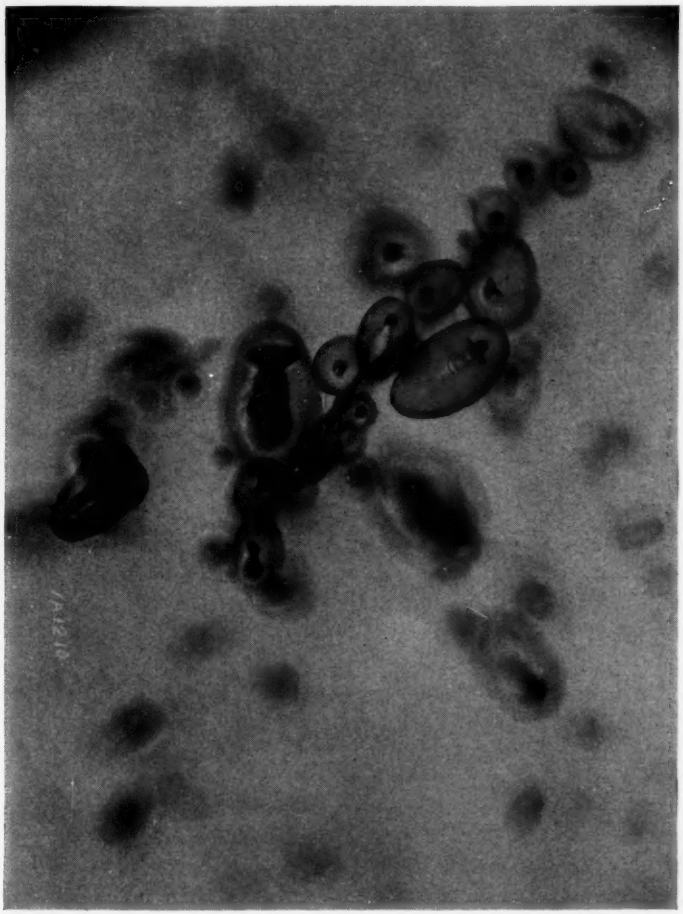

FIG. 19. Photomicrograph of starch grains showing the structure sketched in Fig. 2.

hyde is further increased, the blue-staining granules of the clavate structure will appear brown or black and one can observe that they are arranged in definite layers as shown in Figs. 5, 10, and 20. These brown-black staining particles appear under high magnification to

be in the form of tiny crystals. The dome-shaped end of the clavate structure appears to be the enlarged locus of the grain. If the time of exposure to the fumes of formaldehyde is further increased, the clavate structure will disintegrate leaving the brown-black staining particles in irregular arrangement.

The central and outer portions of the starch grain may be stained and simultaneously caused to separate by the following procedure:

1. Place commercial potato starch grains for 27 hours in a staining solution with formalin: commercial formaldehyde, 5 cc.; distilled water, 5 cc.; 65% ethyl alcohol, 2 cc.; safranin 0 (certification No. NS-10 used, dye content 94%), 10 mg.; fast green FCF (certification No. NGf-4 used, dye content 91%), 10 mg.; normal HCl, 0.04 cc.
2. Decant staining solution.
3. Rinse with water.
4. Dehydrate with alcohol. Clear in xylene. Mount in clarite.

During this treatment there will be considerable swelling of the grain and some staining. In some grains the clavate-shaped central portion becomes quite conspicuous, slightly granular, and swollen especially at the locus (Fig. 15). In some of the grains in which the swelling has progressed further, the membrane formed around the grain becomes thinner at the locus end and thicker at the opposite end and stains uniformly red. A layer enclosing the clavate structure dissolves or ruptures and exposes a spherical body containing the locus (Fig. 16). In other grains in which swelling has progressed further, the outer membrane formed around the grain ruptures at the locus end, and the spherical body containing the locus emerges and completely separates from the cone-shaped peripheral part of the grain (Figs. 17 and 18).

When potato starch grains are permitted to float on the surface of water and are examined under the microscope, one can observe within each grain one or sometimes two spherical-elliptical refractive bodies. The size and location of these refractive bodies may vary considerably. Sometimes they are located to one side of the longitudinal axis (Fig. 7), but more frequently are located as shown in Fig. 8. Fig. 9 shows the location of one in a compound grain.

Differential staining of a ring or layer of substance surrounding the refractive body may be obtained by the following procedure:

1. Smear commercial potato starch grains on a slide previously treated with Adam's gelatin adhesive.
2. Treat with a 3% solution of commercial formaldehyde for 10 minutes.
3. Expose to the fumes of commercial formaldehyde, 8 minutes.

FIG. 20. Photomicrograph of starch grains showing structure sketched in Fig. 5.

4. Stain in the hematoxylin solution described above for 24 hours.

5. Rinse with tap water. Dehydrate with alcohol. Clear in xylene. Mount in balsam.

The starch grain will stain light blue, while a conspicuous deep blue ring will appear around the refractive body. Frequently when two starch grains come in contact with each other, a small strand of the deep blue staining material of the ring will pass out thru the peripheral portion of the grain and join with a similar strand passing out from the deep-blue-staining ring in an adjoining grain. The ring of deep-blue-staining material appears to be reticulated (Fig. 11).

If the staining solution described above, containing safranin 0 and fast green FCF, is used instead of the hematoxylin solution, the ring or layer of deep-blue-staining material around the refractive body stains green while the remainder of the grain stains red. If the action of the stain is observed under the microscope, one notes that the green ring is formed very quickly, continues to deepen, finally diffuses into the refractive body and out toward the surface until nearly all of the red color is obscured by the green. (Fig. 14.) Sometimes the green-staining material is forced out thru the peripheral portion in small strands and collects on the outside of the grain in small droplets (Fig. 12).

STUDIES ON POLYCHROME METHYLENE BLUE

I. Eosinates, their Spectra and Staining Capacity

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ABSTRACT.—**The absorption spectra of eosinates of thiazin dyes in water exhibit absorption maxima at the same spectral locations as do the individual component dyes in aqueous solution.**

Commercial samples of Wright's stain showing thiazin absorption maxima between 620 and 660 mμ generally give satisfactory blood stains. Nuclear staining is redder and cytoplasm grayer blue in 620-640 range, and consequently staining of malaria parasites is less satisfactory in that range. The best malaria stains show their thiazin absorption maxima usually between 650 and 660 mμ.

Successive batches of Wright's stain made by the same manufacturer, as well as experimental laboratory lots, may show wide variations in their thiazin absorption maxima and in their staining characteristics.

Hematologists and malariologists have long been concerned with the variability in staining capacity for blood and for malaria parasites of various lots of the eosinates of polychrome methylene blue prepared by the Wright, Leishman, and other methods.

Recently in connection with published studies on the use of the various azures[1] and of their eosinates[2], we made extensive use of the spectrophotometer for the identification of the various methylene blue and azure samples of domestic and foreign manufacture. This method was used also by Holmes and co-workers[3], by Formanek[4] as well as Scott and French[5] on supposedly purified azures.

[1]Roe, M. A., Lillie, R. D., and Wilcox, A. 1940. American azures in the preparation of satisfactory Giemsa stains for malaria parasites. Pub. Health Rep., **55**, 1272–8. 1941.

[2]Roe, M. A., Wilcox, A., and Lillie, R. D. 1941. Eosinates of the azures and methylene blue in preparation of a satisfactory Giemsa stain from dyes of American manufacture. Pub. Health Rep., **56**, 1906–9.

[3]Holmes, W. C. 1928. The spectrophotometric evaluation of mixtures of methylene blue and trimethyl thionin. Stain Techn., **3**, 45–8.

Holmes, W. C., and Snyder, E. F. 1929. The atmospheric oxidation, or dealkylation, of aqueous solutions of methylene blue. Stain Techn., **4**, 7–10.

[4]Formanek. 1908. Untersuchung und Nachweis organischer Farbstoffe. Berlin. Cited from Scott and French.

[5]Scott, R. E., and French, R. W. 1924. Standardization of biological stains. Mil. Surg., **55**, 229–43 and 337–52.

It does not, however, seem to have been applied to the study of the precipitated and dried eosinates. Preliminary trials showed that a solution of Wright's stain (NWr-10) in C. P. methanol diluted with the same solvent about 1,000 times gave a clearly defined absorption spectrum with absorption maxima at 525 and 648–650 mμ. An unpolychromed methylene blue eosinate diluted in the same way in C. P. methanol gave maxima at 525 and 652 mμ, and diluted in 95% ethyl alcohol at 528–530 and 654 mμ. As the parent lots of eosin Y (LE-11) and methylene blue (E&A old) gave maxima in 95% alcohol at 531 and 656 mμ respectively, this method of exploration was thought worthy of further trial.

Next it was found that dilutions of methanol and methanol-glycerol solutions of thiazin eosinates in distilled water to give final dilutions between 5 and 10 γ per cc. were sufficiently stable to give quite constant readings on the spectrophotometer at any given wave-length over a period of several hours.

TABLE 1. ABSORPTION MAXIMA OF THIAZINS AND THEIR EOSINATES IN WATER*

Graph	Dye and Lot Number	Absorption Maxima in mμ
1	Methylene blue, E. & A., Lot I-K	664
63	Eosin Y, LE-11	517
68	Methylene blue eosinate, Lot I-K (E. & A.+LE-11)	517, 663
72	Azure A, NAz-6	618–620
70	Azure A eosinate (NAz-6+LE-11)	517, 612–622
73	Azure B, NAC No. 9348	652
71	Azure B eosinate (NAC No. 9348+LE-11)	517, 653
85	Polychrome methylene blue, Lot IV EA	652
90	Polychrome methylene blue eosinate (Lot IV EA+LE-11)	517, 652
87	Polychrome methylene blue, Lot IV EB	652–658
91	Polychrome methylene blue eosinate (Lot IV EB+LE-11)	517, 652–660
22	Polychrome methylene blue, Lot IV B	653–658
92	Polychrome methylene blue eosinate (Lot IV B+LE-11)	517, 652–657

*Eosinates were dissolved 0.1 g. to 80 cc. C. P. methanol and diluted with distilled water to give 25 to 30% transmittance at 517 mμ on the Coleman spectrophotometer, usually about 1:200. Readings were taken at 10 mμ intervals from 400 to 800 mμ, at 2 mμ intervals for 10–15 mμ to each side of the maxima and at 5 mμ intervals thru the remainder of the 610 to 690 mμ band.

The results of spectrophotometric examination of several lots of methylene blue, of polychrome methylene blue, and of the various azures are presented in Table 1, together with similar data for the eosinates prepared from the same dye samples with the same sample of eosin. Apparently the absorption spectrum of an eosinate in water presents the same absorption maxima as its component dyes do separately in aqueous solution.

Thin blood films from patients heavily infected with *Plasmodium malariae* were prepared and stained with 26 samples of various

Romanowsky stains (0.1 g. to 80 cc. of C. P. methanol), using thruout prior fixation in C. P. methanol at the bedside and staining not over 24 hours later. Undiluted stain (1 cc.) was applied for 1 minute, then 2 cc. of water buffered to pH 6.5 or to pH 7.0 was added and staining continued for 5 and for 10 minutes.

General ratings were made of these films for the effectiveness of the stain for blood morphology and for parasites. In general it was noted that the longer staining interval and the higher pH-level gave denser nuclear staining, heavier neutrophil granules and denser parasite chromatin. The general effects of each stain are tabulated in Table 2 together with spectrophotometric data on the corresponding samples.

Besides the absorption maxima for the eosin and for the thiazin dyes concerned we have included the width and median point of the band in which D, the logarithm of the reciprocal of the transmittance percentage (T), is at or over 90% of its maximum for the thiazin dye. The purpose of the introduction of these latter is to differentiate between samples showing relatively complex polychrome methylene blues and consequently broad absorption bands, and relatively pure thiazins with narrower bands.

In general it is seen that fairly good blood stains were obtained with most samples showing thiazin absorption maxima between 620 and 660 mμ. Stains with maxima above 660 tend to show irregularity of leucocyte nuclear staining, some cells showing the pale blue of methylene blue eosinate stains. Satisfactory staining of plasmodial chromatin and cytoplasm was restricted to a narrower band, between 650 and 660 mμ. The one sample (Balch stain) with a much lower maximum (624 mμ) which gave good parasite staining had a quite wide absorption band whose median was nearly 10 mμ higher than the recorded maximum. This indicates a considerable mixture of azure B (absorption maximum 652) with a predominant azure A (absorption maximum about 620–624).

These findings agree well with the results of previous studies of the azures and their eosinates in relation to malaria parasite staining. (See footnotes 1 and 2.)

It is noteworthy that 3 successive samples of Wright's stains from the same manufacturer showed quite divergent thiazin absorption maxima and staining characteristics: NWr-13, 656 mμ; NWr-14, 627 mμ; and NWr-15, 660 mμ; and similarly, CWr-12 at 660 mμ and CWr-13 at 620 mμ for another manufacturer. Note should also be made in passing of the discordant results with the year-old and fresh solutions of NWr-13, with maxima respectively at 607 and 656 mμ.

TABLE 2. SPECTROPHOTOMETRIC DATA AND STAINING PERFORMANCE OF COMMERCIAL SAMPLES OF ROMANOWSKY STAINS

Graph No.	Stain and Lot	Absorption maxima		Wave-length band of 90% D λ*			Staining performance†	
		Fluorane	Thiazin	Range λ	Width	Median λ	Blood	Paras.
139	Thionin Eosinate HL38, 12–18–40	516	600	587.0–606.9	19.9	596.9	±	±
120	Wr. Biosol., 1935 sol.	515	605	589.9–630.9	41.0	610.4	±	±
126	NWr-13, 11–24–39 sol.	517	607	592.5–621.7	29.2	607.1	±	±
140	Phlox. Tol. Blue, 12–18–40 sol.	530	618	596.4–638.8	42.4	617.6	±	±
122	CWr-13, 6–10–40 sol.	515	620	601.4–641.7	40.3	621.5	+	+
151	Leishm. NAC No. 9034, 5–6–39 sol.	515	622	603.8–637.7	33.9	620.7	++	+
141	Old War pooled, 12–18–40	517	628	602.4–655.0	52.6	628.7	++	+
135	NWr-14, 9–16–40 sol.	516.5	627	606.6–650.4	43.8	628.5	++	±
142	Balch 11–21–38, 12–18–40 sol.	516	624	604.8–662.2	57.4	633.5	++	++
132	McNeal, 5–1–36 sol.	516.5	634	604.3–652.3	48.0	628.3	++	+
134	Leish. Gr. JWM, 12–18–40 sol.	518	633	599.1–662.8	63.7	631.0	±	±
136	NWr-4, 12–18–40	517	635	605.2–660.6	55.4	632.9	+	+
137	NWr-13, 12–18–40 sol.	517	656	617.2–669.8	52.6	643.5	++	++
146	Leishm. BW, 12–20–40 sol.	517	652	630.5–665.9	35.4	648.2	++	+
149	AzIIE Gr. B, 12–20–40 sol.	517	656	636.4–669.0	32.6	652.7	++	++

133	Leish. Gr. P., 12–18–40 sol.	515	656	640.3–668.8	28.5	654.5	++	+ to ++
144	NWr-10, 12–18–40 sol.	517	658.5	640.1–670.2	30.1	655.1	++	+++
145	CWr-12, 12–18–40 sol.	517	660	645.6–671.5	25.9	658.5	++	++
123	NWr-15, 10–18–40 sol.	516	660	641.1–671.5	30.4	656.3	++	++
143	Hastings NAC 8589, 12–18–40 sol.	516.5	660	642.5–671.7	29.2	657.1	++	+++
127	Wright "H-O", 12–16–40 sol.	517	659	648–670	22.0	659.0	++	++
148	AzIIE Gr. A, 12–20–40 sol.	517	661	647.2–671.4	24.2	659.3	+ to ++	+
150	AzIIE Gr. C, 12–20–40 sol.	517	662	648.7–670.9	22.2	659.8	irr. +	+
125	LWr-9, 12–20–39 sol.	516	661	648.0–671.5	23.5	659.7	++	+
138	H-L Co. Recent, 12–18–40 sol.	516	662	648.7–671.8	23.1	660.2	+++	++
147	Leishm. EA, 12–20–40 sol.	517	663	650.0–671.6	21.6	660.8	irr. +	+
161	LWr-10, 1–16–41 sol.	516	664	649.4–671.7	22.3	660.5	++	++

*D is the logarithm of the reciprocal of percentage transmittancy (T) and is expressed thus: $D = \log 1/T$.
λ is the wave length at which maximum absorption occurs.
The wave-length band of 90% D λ is that band thru out which D is at or over 90% of its value at λ.
†In this column symbols signify as follows: ±, poor; +, fair; ++, good; +++, excellent.

TABLE 3. SPECTROPHOTOMETRIC DATA AND STAINING PERFORMANCE OF EXPERIMENTAL BATCHES OF WRIGHT'S STAIN

Graph No.	Lot number and procedure	Absorption maxima		Wave-length band of 90% D λ			Staining performance†	
		Eosin	Thiazin	Range λ	Width	Median λ	Blood	Plasmodia
162	WA open beaker, water bath	516	656	636.4–668.6	32.2	652.5	+++	++
163	WB Erlenmeyer, water bath	517	656	639.1–669.9	30.8	654.5	+++	+++
164	WD open beaker, water bath, neutralized	516	654	625.5–667.8	42.3	646.6	+++	+++
165	WE open beaker, water bath	517	650	613.6–665.6	52.0	639.6	+++	++
166	WF Erlenmeyer, water bath	516	654	630.6–668.7	38.1	649.6	+++	++
167	WG Erlenmeyer, boiled, reflux, neutralized	515	617	601.7–632.0	30.3	616.8	—	—
168	WH open beaker, water bath, neutralized	516	657	628.0–669.0	41.0	648.5	+++	+++
169	WI small closed flask, Arnold	517	656	637.7–669.0	31.3	653.3	+++	++
170	WK large open flask, Arnold	516	625	604.6–662.4	57.8	633.5	+++	+

*D is the logarithm of the reciprocal of percentage transmittancy (T) and is expressed thus: $D = \log 1/T$.
λ is the wave length at which maximum absorption occurs.
The wave-length band of 90% D λ is that band thru out which D is at or over 90% of its value at λ.
†In this column symbols signify as follows: +, fair; ++, good; +++, excellent.

The former is in the zone of the absorption maxima of thionin (598–602), azure C (611) and methylene violet (3 samples at 602, 608 and 610 mμ respectively). In our previous studies none of these three dyes gave satisfactory leucocyte or parasite staining, and the performance of the year-old Wright stain solution is in accord.

To test this variability further, 1 g. samples of the same lot of methylene blue (LA-7) were polychromed 1 hour with 100 cc. of 0.5% $NaHCO_3$, two (WA, WE) in open 800 cc. beakers in a bath of boiling water, two (WB, WF) in Erlenmeyer flasks in a bath of boiling water, two (WC, WG) in Erlenmeyer flasks boiling under a reflux condenser, one (W1) in a closed 120 cc. Erlenmeyer and one (WK) in an open 500 cc. Erlenmeyer in the Arnold steam sterilizer, and two (WD, WH) in 800 cc. beakers in a water bath with subsequent acetic acid neutralization. All were cooled, filtered, and precipitated with sufficient eosin Y to give a light blue solution between the precipitate granules (Lot LE-11). The precipitates were filtered out, dried and dissolved 0.1 g. to 80 cc. C. P. methanol and spectrophotometric studies and staining tests were made as before. The results are presented in Table III.

Lots WC and WG developed precipitates which filtered out. In WC only the filtrate was used and no eosinate precipitate was formed. In WG, 0.35 cc. glacial acetic acid was added to 100 cc. distilled water and the precipitate dissolved in it and then combined with the filtrate before making eosinate. It was necessary to add as much as 80 cc. water each to lots WA, WD, WE and WH to compensate for evaporation. Separate spectrophotometrographic examinations were made of the filtrate and the redissolved precipitate of lot WG. The 90% maximum absorption bands ranged from 585.5 to 621.5 for the filtrate and 600.7 to 635.6 for the precipitate, with medians at 603.5 and 618.1 respectively, and ordinary absorption maxima at 603 and 614–622. These were combined to prepare the eosinate solution of WG.

The variability in results is more evident in the width and median point of the 90% of maximum absorption band for the thiazin component than in the maxima themselves. Thus the four open beaker samples with thiazin absorption maxima at 656, 654, 650, and 657 mμ gave band widths of 32.2, 42.3, 52.0 and 41.0 mμ, and medians at 652.5, 646.6, 639.6 and 648.5 mμ. The two Arnold specimens showed the greatest variation, maxima at 656 and 625, bands of 31.3 and 57.8, medians at 653.3 and 633.5 for the closed and open flasks respectively.

TWO CONVENIENT WASHING DEVICES FOR TISSUES AND SLIDES

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ABSTRACT.—**Two improved mechanisms are described: (1) For washing fixed tissues, a device which utilizes a downward movement of water with a consequent thoro washing is most convenient, because the washing tubes may be removed or replaced instantly. (2) A pan for washing microscope slides, fitted with a valve stem from an automobile inner tube, produces unusually effective washing and accommodates as many as 80 slides at once.**

1. MECHANISM FOR WASHING TISSUES

Altho useful methods of washing fixed tissues have been reported by Turner[2] and Wetmore[3] the device described here is recommended because of its unusual convenience and effectiveness.[4]

Its convenience is due to the extreme ease and simplicity of operation. Once the device is constructed, one needs merely to place the tissue in the glass washing tube and clip into position. The mechanism is very compact, light, and easily handled; and if made from aluminum, will not discolor a porcelain sink with rust.

The effectiveness of the device is dependent upon its principle, which is similar to that of the method suggested by Galigher[5], who points out two distinct advantages over other methods: (1) "*all* the water passes over the tissue," and (2) "the water moves *downward* so that gravity aids rather than hinders the removal of heavy salts from the material."

Construction of the washing device is not a difficult matter nor is it expensive, altho the current need of aluminum for defense purposes makes that material harder to obtain. The mechanism is very sturdy

[1]Contribution No. 57.

[2]TURNER, J. P. A simple apparatus for washing Protozoa. Science, **74**, 611–17. 1931.

[3]Wetmore, R. H. The use of celloidin in botanical technic. Stain Techn., **7**, 37–62. 1932.

[4]Since this paper was sent to press a somewhat similar apparatus was described by Kersten, H., and Smith, G. F. An apparatus for washing tissue. Stain Techn., **16**, 157–64. 1941.

[5]Galigher, Albert E. The Essentials of Practical Microtechnique. Albert E. Galigher, Inc., Berkeley, Cal. 1934. (See pp. 78, 79).

and should last indefinitely with the possible replacement of a clip after long usage. One needs only an aluminum baking pan $5\frac{1}{2} \times 10 \times 3$ in., a piece of $\frac{1}{4}$ in. aluminum tubing 12 in. long, one piece of angle aluminum 12 in. long, about 48 in. of strip aluminum, and 15 copper rivets with their washers. Small machine bolts might be substituted for the rivets altho, unlike the copper, they would rust.

Details of construction are indicated in figures 1, 2, and 3. Figure 1 is an end view showing the way in which the aluminum tube (t) is held to the arched support (su) by a fitted strip riveted underneath. It also shows the relationship of the angle piece (an) to the clamps (cl) which hold the glass tubes (g). The same feature is shown from top view in figure 3. The angle aluminum is bent over the ends of the pan and riveted in position. By twisting a loop of fine stainless wire around the hilt of the clamp (fig. 3, w) the springiness is adjusted and preserved. Several small holes are punched in the metal tube with a large darning needle after the glass tubes are placed in position so that the holes will be sure to lie immediately over the latter. It is helpful also to place the holes at such an angle that the water is thrown toward the periphery rather than the center of the glass tubes. One end of the aluminum tube is connected to a water spigot by means of rubber tubing (r), while the other end is closed by bending over tightly or by inserting a cork.

The mechanism, as shown in figure 2, will accommodate five glass washing tubes simultaneously, a number which is adequate for the usual laboratory needs or even for small classes in technic. The glass tubes, which are 1 in. in diameter and 2 in. long, may be made of glass tubing or from shell vials with the bottoms removed. They should have both ends spread slightly like the bulb end of a medicine dropper; the upper end to guard against possible slipping thru the clamp, the lower end to hold the silk bolting cloth or cheese cloth which may be held in place by a string or rubber band. The method may be adapted to any size of material merely by changing the mesh of bolting cloth.

The glass washing tubes can be removed or replaced quickly and easily and may be adjusted to any height desired. When they are raised nearly to the aluminum tube, the fine spray of water from the holes in the latter does not strike the tissue with any force and yet gravity causes a constant and complete change of water around the tissues.

2. Washing Pan for Slides

Extreme simplicity, convenience and effectiveness characterize the pan designed for washing microscope slides.

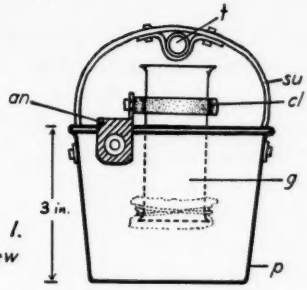

FIG. 1. End View

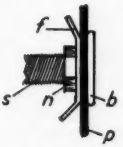

FIG. 4.

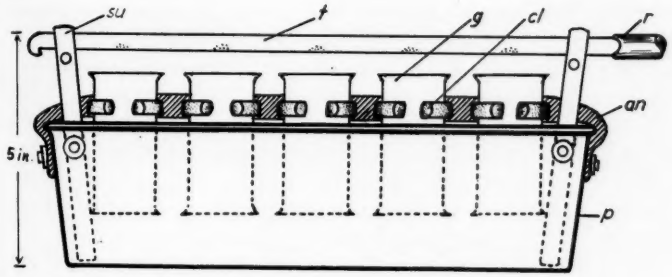

FIG. 2. Side View

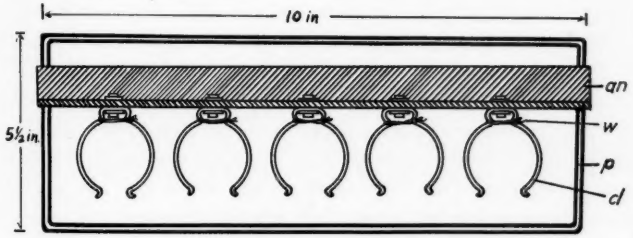

FIG. 3. Top View

FIGURES 1–3. Device for washing fixed tissues.

an. Angle piece
cl. Clamp
g. Glass tube
p. Pan
r. Rubber tubing
su. Arched support
t. Tube
w. Wire

FIGURE 4. Details of construction of pan for washing slides.

b. Base
f. Flanged washer
n. Nut
p. Wall of pan
s. Valve stem

An aluminum baking pan ($5\frac{1}{2} \times 10 \times 3$ in.) is fitted at one end near the bottom with a valve stem from a discarded automobile inner tube. The old style stem with a nut that screws on the outside is necessary. The base (fig. 4, b) and flanged washer (f) have a groove and ridge arrangement which make a water tight joint with the aluminum pan (p) when the nut (n) is screwed down tightly. The valve stem proper (s) should be sawed off to about $\frac{3}{4}$ in., leaving just enough for a good junction with rubber tubing to the water spigot.

A pan of this size will accommodate two large monel metal slide staining racks[6] holding 25 slides each or eight of the smaller racks which hold ten slides each. If one uses, for stains and alcohols, tall Stender dishes into which the small racks fit, he can dispense with practically all handling of individual slides. Placing slides in the rack (after affixing the sections), examining one or two in order to control differentiation, and finally mounting under cover glasses constitute the only individual handling necessary; thus the staining time is cut down considerably.

The washing is unusually effective since water enters the pan from the bottom, passes directly around and between the slides and finally flows over the rim of the pan.

NOTE.—Numerous advantages of aluminum over other construction materials are obvious, but since aluminum is now hard to get, some substitutes might be suggested. Tin baking pans of the same size are available at any five-and-ten-cent store but they would have to be covered with varnish or enamel or asphalt paint to prevent their rusting. Aluminum enamel is the best coating but it is as scarce as the metal itself. Brass or copper tubing could be used instead of aluminum tubing but would necessitate the use of a drill in place of a darning needle for making the holes. Instead of the strip aluminum used for the supports and clamps, one could substitute either copper, galvanized iron, or tin-plate, altho the latter would have to be protected from rusting. Soldered joints could replace the rivets with any of these substitute materials.

[6]Central Scientific Co. catalogue numbers 48060 and 48070, monel metal staining racks for holding microscope slides for staining by immersion.

TECHNIC FOR PHOTOGRAPHING EARLY CLEAVAGE STAGES OF THE HEN'S EGG

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Altho photographs have been secured of early cleavage stages in the pigeon's egg, no photographs of corresponding stages of development of the hen's egg have been published. Satisfactory photographs of early cleavage stages of the hen's egg have been difficult to obtain in the past because of the indistinctness of the furrows and the lack of contrast between them and the remainder of the blastodisc.

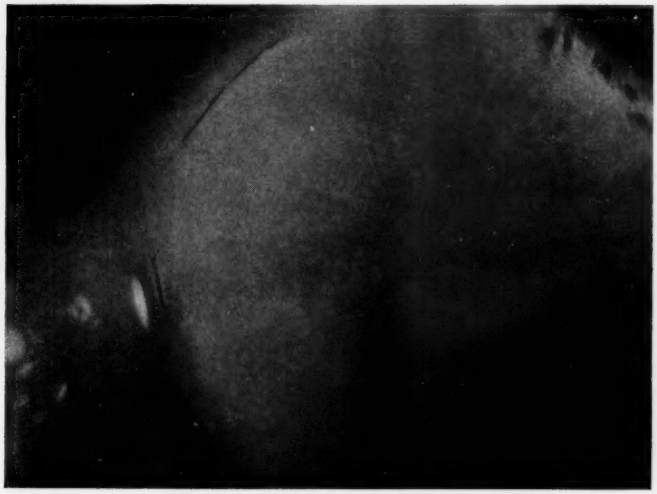

Fig. 1. Photograph of an unstained blastodisc of a hen's egg showing an 8-cell cleavage stage.

The indistinctness of the cleavage furrows in the hen's egg as compared with those of the pigeon's egg was recognized by Patterson (1910) who, after having studied both types of eggs, states (p. 109): "The lack of a furrow is the cause of the indistinctness of the early cells. In this respect, the early cleavages of the hen's egg differ greatly from those of the pigeon's egg, for in the latter their clearness is such as to permit photographing the living cells, while in the former photographs are impossible, except in a few cases".

A number of the photographs of early cleavage in pigeon eggs published by Blount (1909) and reproduced by Lillie (1919) were obtained of the living egg, one of which was taken thru a window in the shell, while the others were secured after the intact yolk was immersed in normal saline solution. Other photographs published by Blount were taken after the blastodiscs were killed and while the yolks were immersed in the killing fluid. Still other photographs were taken from whole-mount preparations, but the author makes no mention of either removing the vitelline membrane or staining the blastodisc before photographing.

During a recent study of the early development of the embryo of the domestic fowl, a technic was devised which makes it possible

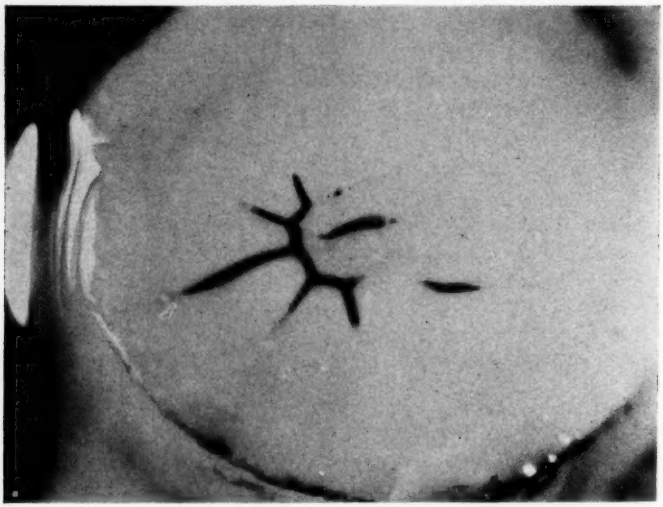

FIG. 2. Photograph of the same blastodisc as shown in Fig. 1, stained with gentian violet before photographing.

to study and to photograph whole-mount preparations of early cleavage stages of the chick embryo. The following procedure was used in preparing the tissue for photographing.

The entire yolk of the egg containing the blastodisc to be photographed is placed in Bouin's fixative and allowed to remain in this solution from 24 to 48 hours. It is important that all the albumen adhering to the vitelline membrane about the blastodisc be removed before fixing the yolk; otherwise difficulty will be experienced in locating the blastodisc. After fixing for 24 to 48 hours, the yolk is

removed from the fixative and placed in 60% alcohol. The vitelline membrane is then removed by means of small forceps. If care is taken in removing the vitelline membrane, the blastodisc is exposed and left undamaged on the yolk. The blastodisc is now removed by cutting about the periphery and beneath the disc with a small pair of scissors. The blastodisc, after being removed from the yolk, is placed in the cell of a depression slide. Two or three drops of 5% gentian violet[1] in 95% alcohol are then applied to the upper surface of the blastodisc. The tissue is then destained by the application of several changes of 60% alcohol. If the destaining process is done carefully, a point can be reached where the stain will be completely removed from the upper surface of the cells, leaving only a small amount of stain in the cleavage furrows. This creates a marked contrast between the cells and the cleavage furrows so that they can be easily photographed. If the destaining is carried too far, the tissue can be restained and the destaining operation repeated.

Photographs of the same blastodisc of a hen's egg before and after preparation by the method just described are shown in Figs. 1 and 2. It will be observed that where the special technic was used (Fig. 2) the cleavage furrows stand out sharply and in marked contrast to those found in the unstained balstodisc and shown in Fig. 1.

After photographing, the blastodiscs can be preserved as semi-permanent preparations by replacing the 60% alcohol used in the destaining process with Canada balsam.

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BLOUNT, M. 1909. The early development of the pigeon's egg with especial reference to polyspermy and the origin of the periblast nuclei. Morph., **20**, 1–64.

LILLIE, F. R. 1919. The development of the chick. *2nd Ed.* Henry Holt and Co., New York.

PATTERSON, J. T. 1910. Studies on the early development of the hen's egg. I. History of the early cleavage of the accessory cleavage. Morph., **21**, 101–34.

[1]Coleman and Bell's "gentian violet, improved" (a methyl violet 2B) was employed in this work.

A SIMPLE METHOD OF TRANSFERRING TISSUES

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It is a common practice among histologists while preparing tissues for sectioning to transfer the tissues from one reagent to another by

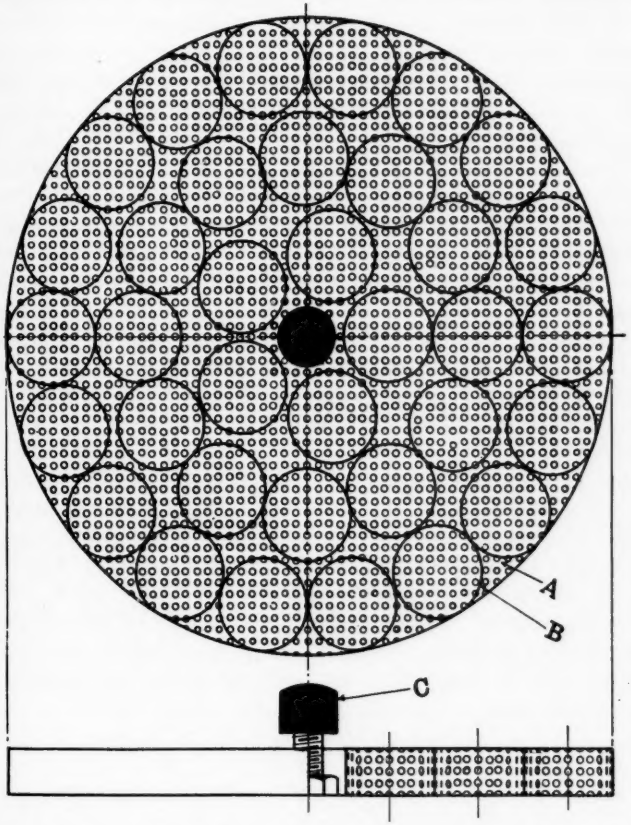

Fig. 1. Carrier used for transferring tissues. It is composed essentially of a base plate (a), individual tissue chambers (b), and a handle (c).

means of small forceps or a spatula. This method can be used safely when the type of tissue being studied is of such a nature that it will

not be injured by these operations. When, however, the tissue is fragile, extreme care must be exercised, if the above method is employed, to prevent damage. In a recent study of maturation and and fertilization of the hen's egg, difficulty was experienced due to breakage of blastodiscs while transferring them from one reagent to another. In order to reduce the chances of damage and to facilitate and simplify the operation of transferring these fragile tissues, the tissue carrier described in the following paragraph was designed.

The tissue carrier is shown in the accompanying illustration. It consists essentially of a perforated metal plate, A, 3½ inches in diameter, to which are soldered 35 perforated circular metal bands. Each band is ½ inch in diameter and ¼ inch in depth and forms with the base plate the individual tissue chamber, B. The base plate and the bands are made of number 30-gauge monel metal having 225 or more perforations per square inch. Other perforated metals may be substituted for the monel metal but in such a case it is advisable, after the carrier has been constructed, to have it plated with some non-corrosive metal such as silver, chromium, or cadmium. A small, wooden knob, C, attached to the head of a ⅛-inch brass bolt serves as a handle. The bolt is screwed into a nut which is soldered to the upper surface and at the center of base plate A. The depth of the tissue carrier, including the handle, is $\frac{7}{16}$ inch. The entire carrier will fit into a 4-inch Petri dish, which can then be covered so as to prevent evaporation of the reagent being used.

The tissues to be dehydrated, cleared, and infiltrated are placed in the small perforated chambers. Once the tissues are placed in their respective compartments they need not be handled individually again until the time of embedding. In order to identify individual tissues, a small slip of paper labeled with laboratory ink is placed in each chamber. To transfer the tissues the carrier is lifted from one solution and placed in a second Petri dish containing the next reagent. This method greatly reduces the chances of damaging the tissues and makes it possible to transfer a large number of tissues quickly and easily with one operation from one reagent to another.

THE PLASTIC ETHYL METHACRYLATE IN ROUTINE LABORATORY TECHNIC

MARY S. CARBONE AND DONALD J. ZINN, *Osborn Zoological Laboratory, Yale University*

ABSTRACT.—**The use of ethyl methacrylate as a permanent, transparent, colorless medium for mounting *in toto* invertebrate and vertebrate embryos and small adults is demonstrated.**

The liquid monomer is partially polymerized by heating over a hot-plate, the reaction being aided by the use of benzoyl peroxide as a catalyst. The object to be mounted is fixed, stained, dehydrated, and cleared in the usual manner, and is then infiltrated with the partially polymerized ethyl methacrylate. It is oriented on a hardened base in a glass or porcelain dish, and the mold is then allowed to harden (polymerize) completely at a temperature of 38–40° C. under a 250-watt infra-red, ultra-violet bulb. After the block is removed from the dish it may be polished by the ordinary metallurgical methods. More than 40 species of animals have been satisfactorily treated in this manner.

The great need for a satisfactory method of mounting various small invertebrates and vertebrate adults and embryos, as well as transverse or longitudinal sections too large or too thick for mounting on ordinary slides, has long been felt. Of the few technics that have been mentioned in the literature, there appears little doubt that the best is to employ ethyl methacrylate, a resinous plastic soluble in absolute ethyl alcohol, ether, dioxan, oil of wintergreen, xylene, and most soluble in chloroform. The use of a similar material, methyl methacrylate, has been suggested by Hibben and by Cole[1], while Puckett[2] has worked with ethyl methacrylate, but neither of these authors has demonstrated the apparent general application of these plastics to all small, cleared metazoa. There are, in addition, certain refinements in technic developed by the authors which have simplified the process and made the creation of satisfactory clear blocks more certain.

[1]Cole, Elbert C. 1938. Methyl methacrylate as a laboratory tool. Science, **87**, 396–8.

Hibben, J. H. 1937. The preservation of biological specimens by means of transparent plastics. Sci., **86**, 247–8.—A recent personal communication from this author indicates that he has now worked out a more complete method for mounting specimens in methyl, ethyl, and butyl methacrylates.

[2]Puckett, W. O. 1940. Ethyl methacrylate as a mounting medium for embryological specimens. Science, **91**, 625–6.

The advantages of a permanent, transparent, colorless, easily prepared mounting medium are considerable (a) from the point of view of the teacher and the museum preparator, (b) for the personal use of the student in the laboratory and (c) for the permanent museum demonstration. The problems of the storage of specimens, cost of slides, jars and other containers disappear, and the potential breakage and color-changes of the preserving fluids are virtually negligible. Whelan[3], in his comprehensive review of the use of various plastics in entomology, states with regard to the methacrylate resins—"The solid form possesses extraordinary colorless transparency, stability against aging, and chemical resistance to many reagents". The advantages of ethyl methacrylate (Plexiglas), manufactured by the Röhm and Haas Company, Philadelphia, Pa., ($6.00 per gallon) are demonstrated in the greater ease with which it may be handled in the preparatory and in the hardening processes.

The liquid monomeric ethyl methacrylate is now shipped in a pure state; i.e., it does not contain an inhibitor (Puckett, op. cit.). Shipment in dry ice and keeping the material in a refrigerator at a very low temperature prevent spontaneous polymerization. Puckett, as well as Sando[4], worked with material which was kept in the monomeric state by means of an inhibitor, hydroquinone, which either had to be washed out with KOH, or had to be separated by distillation. Since it was found possible to keep the liquid resin as a monomer by means of refrigeration, the use of an inhibitor was eliminated by the manufacturer. And because light and heat aid in spontaneously polymerizing the monomeric ethyl methacrylate, only the amount of material which is to be used should be prepared.

Our procedure is as follows:

To 500 cc. of the monomer in a liter Florence flask are added 50 cc. of a catalytic solution, made by dissolving 5 g. of benzoyl peroxide in 100 cc. of the monomer. The flask is stoppered with a cork thru which a thermometer has been inserted, as well as a glass L-tube drawn to a fine point to allow for dissipation of heat generated in the polymerization reaction. An electric hot-plate is used rather than a water-bath or any device which necessitates the use of a flame, as the material is inflammable, and its polymerization may easily get out of control. To prevent too rapid a polymerization, the temperature of the hot-plate is adjusted so that the methacrylate solution will be between 83° and 85°C. While the reaction is taking

[3]Whelan, Don B. 1941. The role of plastics in the field of entomology. Kansas Entom. Soc., **14**, 73–89.

[4]Sando, Charles. Embedding Specimens in Plexiglas. (Dist. by Rohm and Haas Co., Phila., Pa.)

place, the flask is shaken every few minutes in order that excess heat be liberated, and thus boiling over of the solution is prevented. At 85°C. the methacrylate becomes as viscous as syrup. When this point is reached, the flask is uncorked and plunged into ice-water. After having cooled, the flask is well corked, and stored in the refrigerator until the solution is needed.

Both vertebrates and invertebrates have been mounted in this medium: chick, pig and rat embryos, turtles, frogs, fish, tunicates, amphioxus, bryozoa, hydroids, flatworms, roundworms, brittle-stars, molluscs, spiders, 41 species in all. These animals are fixed in alcohol, formalin, or other fixatives; and then stained, dehydrated, and cleared as for ordinary mounts.

Dishes made of glass, ceramics, tin, and brass may be used as molds. The glass and ceramic containers prove best from the point of view of: (1) the ease with which the polymerized methacrylate was removed from the mold, and (2) the finish of the block. The molds, half-filled with the partially polymerized solution, are tightly covered, with cork or glass, and placed in an oven at 50°C. for 24–48 hours. This final heating causes the ethyl methacrylate to be polymerized completely, and forms a hard base on which to mount the object. During this process, the solution evaporates about 50%.

The animal to be embedded is placed in a dish of the partially polymerized material, which in turn is set in a desiccator for 12-24 hours to allow for evaporation of the clearing agent and to permit infiltration. Just before placing the animal in the mold, the dish is filled with the partially polymerized material and then allowed to stand so that the fresh solution will slightly soften the base. This step serves to facilitate orientation, since the object may move during the final heating if it is not held fast in the softened surface of the base. A small India ink label may now be included.

The mold should be treated in either of two ways. The better method for the final polymerization reaction involves the use of a strong infra-red, mild ultra-violet bulb.[5] The bulb is suspended about 10 inches over the dish, which is kept slightly uncovered. The heat generated by the lamp reaches 38°–40°C., and the length of time required to harden a block depends upon its size. For example, a block 6.5 cm. in diameter and 1.5 cm. thick will harden in 20 hours. The other technic requires that the methacrylate be finally polymerized in an oven at 50°C. The authors prefer the former method because the block can be watched more readily during the polymerization.

[5]General Electric Mazda CX, G-30; 250 w., 120 v.; Strong infra-red, mild ultra-violet; 4750 Lumens; $1.25.

After the block has hardened, and while the dish is still warm, the edge of the mold is freed from the walls of the dish with a knife, and the dish is plunged into icewater or put into the refrigerator until the entire block is loosened.

The molds are easily shaped and polished. The final shape of the block may be formed with a hacksaw. A flat machinist's file is used to remove deep surface cuts, and a finer file (magneto file) is employed to smooth this surface. Final polishing is accomplished on a revolving wheel by using a simplified metallurgical polisher with fine polishing alumina such as that manufactured by the Fisher Co.[6]

If air is trapped in the object or in the block during the final polymerization, or if a thick froth forms which will harden when not noticed in time, the specimen may be rescued by dissolving the polymer in chloroform, and the embedding procedure repeated.

Because of the nature of the final polymerization of the plastic, there are certain conditions under which particular caution must be observed. This concerns especially the embedding of soft-bodied invertebrates such as those of the genera, *Lumbricus*, *Dolichoglossus* and *Placobdella*, in which the epidermis is sometimes pulled apart. The authors were not able to correct this. In addition, slight shrinkages were noted in the exoskeletons of several species of the Arachnida. Of the stains successfully used during this experiment, borax carmine and Feulgen tended to fade slightly. Colored clearing agents such as beechwood creosote were found unsatisfactory, as they imparted a yellow cast to the finished block.

[6]Fisher Polishing Alumina, Grade No. 3, Fisher Scientific Co., Pittsburgh, Pa.

A SCHEDULE FOR CHROMOSOME COUNTS IN SOME PLANTS WITH SMALL CHROMOSOMES

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ABSTRACT.—**A schedule is given which produced excellent results for somatic chromosome counts on some British species of *Galium*, These species present great difficulties owing to the small size of the chromosomes (ca. 1.5μ long), the large somatic numbers (up to 96) and the slenderness of the root tips. Special features of the schedule, which is the result of much experiment with various technics, are: fixation with Belling's Navashin type fixative, which was quite the best tried; the modification of Randolph's card mount method to overcome the difficulty of the small diameter of the root tips by mounting together a number from the same plant, so that they can be embedded and sectioned almost as easily as much larger root tips; staining with dilute (0.1%) crystal violet, the most critical stain used, after mordanting with 1% aqueous chromic acid to intensify the stain in the small chromosomes; and the addition of an extra stage of differentiation in absolute alcohol diluted with xylol to remove strands of stain, which are often left in the cytoplasm between the chromosomes, since clove oil, usually the last differentiating fluid used, differs from alcohol in removing crystal violet more rapidly from the chromosomes than from the cytoplasm. It is suggested that this schedule will be valuable in chromosome counts of other plants where similar difficulties arise.**

The British species of *Galium* have very small chromosomes (ca. 1.5μ long) whose number may be as high as 96, and they therefore present great difficulties for the cytologist. There is need for the utmost care in fixation owing to the high magnification that must be employed, and also to the large number of chromosomes commonly found in these species; and there are also difficulties in bringing about an adequate staining of the chromosomes. A further source of trouble is that the root tips, which were the only suitable parts of the plants for chromosome counts at the time the work was done, are of very small diameter, being little thicker than hairs when growing in soil.

Among numerous fixatives tried, Belling's Navashin type fixative

[1]Present address: Imp. College of Trop. Agriculture, St. Augustine, Trinidad, B.W.I.

(formula in La Cour, 1937, and used with the larger proportion of formaldehyde) was the only one to give excellent results. The chromosomes stained well, were usually well spaced, and were slightly swollen and shortened, a fact which facilitated counting. After Randolph's CRAF, the chromosomes were longer, often with a tendency to clump together, and were less intensely stained. Medium Flemming's fluid resulted in a still more inferior fixation, altho the chromosomes were swollen and intensely stained, rather as after Belling's fixative. La Cour's 2BE and 2BD gave variable staining, some metaphase plates being unstained, while others, even in the same preparation, were quite well stained. Apart from this serious drawback, 2BE fixation was quite good. Modifications of some of these fixatives were tried, but they either gave similar or inferior results.

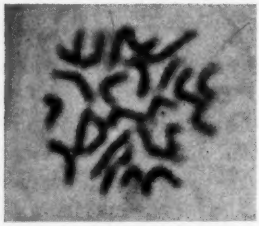

FIG. 1. *Galium palustre* (diploid, 2n=24) treated according to accompanying schedule. × ca. 4000.

The difficulty of the small diameter of the root tips was eased by growing the plants in tap water, which was changed daily to improve aeration. Then considerably stouter and straighter adventitious roots were produced in abundance, especially by the marsh species. Even so, the root tips were so slender that something like 10 or 15 roots had to be sectioned, especially in the case of plants with 96 chromosomes, to be relatively certain of obtaining an excellent metaphase plate. To achieve this without undue labor, all the root tips had to be orientated parallel to one another in the melted wax, but their small size made this rather tricky operation particularly difficult. Accordingly Randolph's card mount method (1940) was modified to obviate these difficulties. His method was followed fairly closely, using for gum Stephen's "strongest mucilage". The root tips were hardened in 50% alcohol and then mounted; a number from the same plant were stuck parallel and close together so that they supported one another by surface tension. They were handled by means of small forceps, and were not dried before mounting, because the small amount of alcohol carried with them was useful for its surface tension effect, and essential to keep the delicate tissues from drying up. Once mounted, it is a simple matter to transfer the whole card from one fluid to another without danger of loss, such as is present when the minute root tips are not attached to one another. Incidentally this method allows economy in dehydrating fluids, since a number of mounts from different plants can be treated

together. The times of dehydration and infiltration should be longer rather than shorter owing to the mass of paper which should also be infiltered.

The staining schedule was modified from Randolph's procedure quoted by Newcomer (1938). Dilute (0.1%) crystal violet was found to give best results, and his mordanting with chromic acid before staining was employed in order to intensify the stain in the small chromosomes. A rather longer time (5 minutes) was given in the alcoholic iodine solution. Iodine treatment of less than 3½ minutes

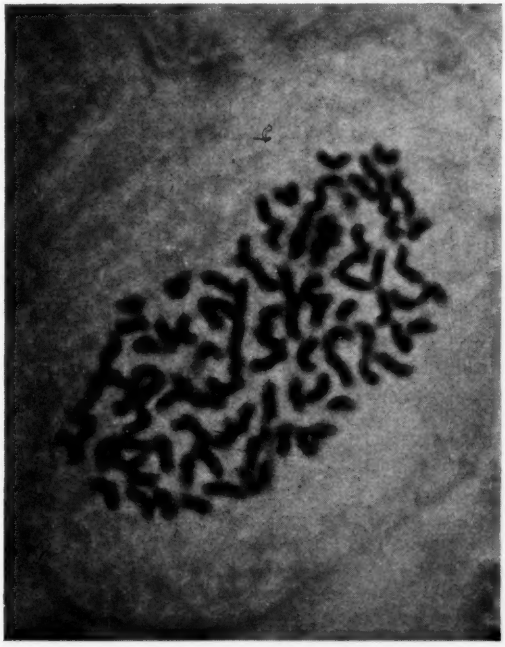

FIG. 2. *Galium palustre* (octoploid, 2n = 96) treated according to accompanying schedule. × ca. 4000.

usually resulted in stain being retained in the cytoplasm, while the chromosomes were, in general, stained lightly after more than 7 minutes in the iodine solution. There was, however, considerable variation in this behavior, suggesting that the optimum time varies with the species and the treatment. An important addition to the schedule was about a minute spent in the mixture of xylol and alcohol; this resulted in the clearing away of strands of stain, which otherwise

were usually left in the cytoplasm between adjacent chromosomes, making interpretation more difficult. These strands of stain appear to be a result of clove oil, since it removes the stain more rapidly from the chromosomes, and it is the last differentiating fluid used. The extra stage in alcohol diluted with xylol removes them without reducing the intensity of the stain in the chromosomes, and therefore increases the contrast.

Other stains tried were Heidenhain's iron-alum hematoxylin and Feulgen. Neither of these was so critical, altho the latter has been used in many different ways, including Warmke's section-smear technic (1941). Some difficulty was experienced in staining the chromosomes with sufficient intensity, but partial success was achieved following his schedule with 5 hours in the stain and 3 minutes in each of the sulphite solutions, while the contrast was improved by 10 minutes in running tap water after staining, as recommended by Warmke. The main drawback to this technic was that pressure stretched out the constrictions, greatly separating small parts of the chromosomes, and making interpretation very difficult. In addition, altho the more evenly spaced chromosomes were easily separated by pressure, groups of overlapping chromosomes, whose interpretation was in doubt, were much more difficult and sometimes impossible to separate.

The following is the complete schedule:

(1) Fix the root tips in Belling's Navashin type fixative. Evacuate for a few minutes and leave	overnight
(2) Harden in 50% alcohol	3 hours
(3) Mount root tips on card	
(4) Dehydrate and harden gum in 70% alcohol	overnight
(5) Continue dehydration, infiltration and embedding in paraffin wax by the chloroform method (La Cour, 1937)	a total of about 10 days
(6) Cut sections (transversely for chromosome counts) about 10μ in thickness	
(7) Dissolve out the wax in xylol, and take the slides down from ethyl alcohol to water in the usual way	
(8) Mordant in 1% aqueous chromic acid	20 minutes
(9) Rinse in tap water and then in two or three changes of distilled water	about 10 minutes
(10) Stain in 0.1% (or 0.5%) aqueous crystal violet	2½ hours
(11) Rinse in tap water	
(12) 1% iodine and 1% potassium iodide in 80% alcohol	5 minutes
(13) 95% alcohol	2–3 seconds

(14) Three changes of absolute alcohol	a total of 30–45 seconds
(15) Clove oil	10–15 seconds
(16) Xylol	2 minutes
(17) Xylol, 2 vol., to 1 vol. absolute alcohol	45–100 seconds
(18) Three changes of xylol	a total of about 2 hours

(19) Mount in Canada balsam.

This schedule is relatively short, as long periods of dehydration are eliminated; the longest part is the infiltration, which should not be much less than 8 days, or the card will not be adequately infiltered. Uniformly good results were obtained on the species investigated with the slightly swollen and intensely stained chromosomes standing out, sharp in outline, from the colorless cytoplasm, even under the magnification of 2,700 diameters which was needed for counting. Little stain was retained by the nucleoli or resting nuclei, so that the chromosomes were easily found and were rarely obscured by overlying cell structures.

It is suggested that this schedule may prove to be of value for other species with small chromosomes where similar difficulties arise, and the modification of the card mount method should be valuable for all plants with minute root tips.

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AN INEXPENSIVE APPARATUS FOR CUTTING TISSUE SECTIONS ON THE SLIDING MICROTOME BY THE "DRY ICE" METHOD

M. V. ANDERS, *Brown University, Providence, R. I.*

The use of "dry ice" in the preparation of frozen sections in histological technic has been increasing in popularity in recent years. Current literature largely deals with apparatus adapted for cutting large blocks of tissue, such as transverse or longitudinal sections of whole brains or other large organs, and usually must be constructed

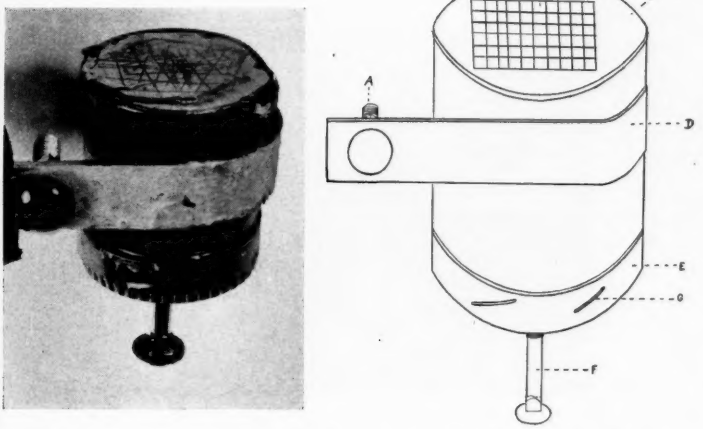

FIG. 1. Photograph and sketch of carrying unit for the "dry-ice", showing the details of construction.

(A) Mounting screw for attaching freezing unit to the microtome micrometer-screw rack.

(B) Piece of grooved tin acting as a freezing surface for the tissue to be cut.

(C) Bottom of can filled to the edges with solder.

(D) Angle iron soldered to side of can for carrier attachment.

(E) Can lid.

(F) Stove bolt acting as plunger to force "dry-ice" up against the bottom of the can for the freezing operation.

(G) Slots and grooves for keeping can lid tightly in place.

as a unit or in part by some particular manufacturer. The apparatus used by the author resembles that used by Dr. J. W. Lind-

say, M.D.[1], but is less expensive and more easily constructed. The necessary materials may be found around the laboratory or may be purchased locally for not more than the total cost of twenty cents.

The container for the "dry ice" is a small tin can, the size of which is governed by the type of work to be done. The one in use at present (Fig. 1) was the container for small lock washers purchased at the local 'dime' store. It is 1¾ inches in diameter by 2 inches high, with a screw-on top. The bottom is strengthened and adapted to act as a freezing surface by coating the outside with a heavy layer of solder, in which is fused a small piece of tin properly grooved to receive the block of tissue. Both the piece of tin and the surrounding solder are made to form a flush surface with the edge of the can. The cap of the can is pierced thru the center by a hole just large enough to carry a 1⅛ inch stove bolt. The nut of this bolt is soldered to the

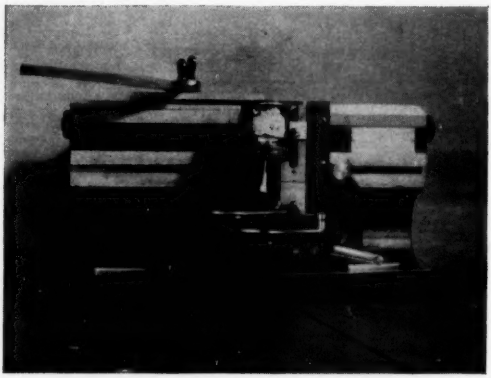

FIG. 2. Freezing unit mounted on a Bausch and Lomb sliding microtome ready for use.

inner surface of the cap to match the hole, permitting the bolt to be screwed thru the cap and adjusted from the outside of the can. A piece of tin or cork may be attached to the end of the adjustable bolt, thus providing a means of continually forcing the "dry ice" up against the surface on which the tissue is to be frozen.

To attach this container to the microtome, a two-inch angle iron may be bent so as to fit snugly around the circumference of the can and soldered in place close to the cap-end. This strap of iron is allowed to project tangentially 1¼ inches from the side of the box,

[1]Lindsay, J. W. 1931. Frozen sections prepared by use of "dry ice". J. Amer. Med. Assoc., 96, 773.

the side from which it projects being governed by the type of microtome for which it is made. It should be made to hold the base of the can upward, or facing the entering edge of the microtome knife. A small stove bolt, selected with the head the correct size to fit the slide-groove of the object-holder of the microtome on which the apparatus will be used, is then put thru the hole which is already in the free end of the projecting strap of iron, and the apparatus is ready for use.

The celloidin-block carrier of the sliding microtome is removed and the "dry ice" apparatus inserted in its place (Fig. 2). The box is filled with small pieces of "dry ice", the stove bolt tightened till the "ice" is in contact with the freezing surface, and the tissue is put in place.

Good thin sections of the spinal cord of small laboratory animals have been obtained consistently with the above apparatus. Both gelatin-embedded tissues and non-embedded tissues have been sectioned equally well. Between one hundred and two hundred sections have been cut with one pound of "dry ice", at the initial cost of but six cents. This is decidedly more economical than the use of liquid carbon dioxide. Good results have been obtained without the use of any insulation, but by lining the box with a thin layer of felt or paper toweling, the apparatus becomes even more economical. If desired, freezing may be hastened by placing small pieces of "dry ice" around the tissue-block for the first minute or two.

An additional advantage of this method is the small amount of space required with the microtomes which are usually available. The sliding microtome has the advantage that the angle of entrance of the blade to the block of tissue may be varied to obtain good sections from tissues of different consistencies. In the case of soft tissues, thinner sections may be cut by the use of a small basket, constructed of a piece of thin wood with a wire clip on either end, which is clamped over the back edge of the microtome knife to carry a piece of "dry ice" to cool the blade.

NOTES ON TECHNIC

Simultaneous Staining with Sudan-Hematoxylin

Mixtures of 70% alcoholic Sudan III with Delafield's or Ehrlich's hematoxylin have been found to stain fat, nucleus and cell structures simultaneously. They keep well and maintain their staining qualities for 6 months or more provided they are prepared with freshly made Sudan. The precipitate appearing after mixing of the two liquids must not be removed.

The method gives good results and has the advantage of being simple and quick. The time can be reduced still further by using ammonia-water (approximately 1 drop of ammonia to each 5 cc. of tap water) for differentiation, a matter of importance for clinical work in rush diagnoses. The risk of losing mounted sections, often encountered with successive staining, is reduced to a minimum.

The sections are brought into the mixture from distilled water or weak alcohol. After staining, they are transferred to water for differentiation. No weak alcohol is required after staining if this is done in a well closed vessel.

The proportions of the mixture depend on the time required for staining the fat. Where 15-minutes' staining is needed, 5 parts of Sudan III with 1 part of hematoxylin have been found satisfactory. Many fats stain within 5 minutes, which enables a considerable shortening of the whole procedure. For such cases a mixture of 3 parts Sudan III with 1 part hematoxylin is required. The procedure is as follows:—

1. Stain in Sudan-hematoxylin..... 5 minutes.
2. Differentiate in ammonia-water..... 15–20 seconds.
3. Tap water..... 15–20 seconds.
4. Enclose in glycerin or glycerin-jelly.—Emil Liebmann, *Dazian Foundation Fellow, Division of Microscopic Anatomy, Tulane University, New Orleans, La.*

An Improved Acid Hemalum Formula

Mayer's acid hemalum has the advantage over some other alum hematoxylin formulae in its ease and speed of preparation and selectivity of nuclear staining; but solutions are rather promptly exhausted and do not keep particularly well. Increasing the concentration of hematoxylin and adding glycerol to the formula appear

to have remedied these defects. The old and new formulae are as follows:

	old	new
Hematoxylin	1.0 g.	5 g.
$NaIO_3$	0.2 g.	1 g.
$AlNH_4(SO_4)_2+12\ H_2O$	50.0 g.	50 g.
Distilled water	1000.0 ml.	700 ml.
Glycerol	—	300 ml.
Glacial acetic acid	20.0 ml.	20 ml.

The alum is first dissolved in the water, then the hematoxylin, then the iodate is added, and, when the color change has occurred, then the glycerol and the glacial acetic acid. No ripening is necessary.

Very satisfactory staining may be attained in as little as two minutes on ordinary formaldehyde-fixed human surgical material, but a 5-minute interval is usually employed. Bluing for 2–10 minutes in tap water and counterstaining one minute in 0.2% aqueous eosin Y are followed by dehydration in alcohol as usual.

Hematoxylin solutions several months old are apparently as good as fresh. It has not yet been determined just how long it will keep.

This formula and the Weigert acid-iron-chloride formula were recently employed in the testing of 14 different lots of hematoxylin in comparison with the Delafield formula and with Mallory's phosphotungstic acid hematoxylin. All four formulae gave satisfactory results with the same 12 lots and unsatisfactory with the other 2 lots. Delafield formulae were used after 6 and 8 weeks' ripening. Phosphotungstic formulae required 20 weeks for fully satisfactory ripening.

The hematoxylin samples used were the American certified lots FH-10, FH-11, FH-13, FH-14, FH-15, FH-16, FH-17 and FH-18, an uncertified lot from Harmer about 1918–1920, the German lots E&A No. A20–385, E&A No. B115 and Grübler No. 11.38, all of which were satisfactory and Grübler 04.9 and Am. Dyewood No. 251, 1917, which were unsatisfactory.—R. D. Lillie, *Senior Surgeon, USPHS., National Institute of Health, Bethesda, Md.*

LABORATORY HINTS
FROM THE LITERATURE

A DEPARTMENT DEVOTED TO ABSTRACTS OF BOOKS AND PAPERS FROM OTHER JOURNALS DEALING WITH STAINS AND MICROSCOPIC TECHNIC IN GENERAL

MICROSCOPE AND OTHER APPARATUS

KOGA, ARATA, and AMANO, S. **Ultraviolettmikroskopie in der Pathologie. III.** *Trans. Soc. Path. Jap.*, **30**, 115–20. 1940.

This review is based on the use of a microscope with a quartz lens system adapted for the 2750Å cadmium or the 2800Å magnesium line. Ultraviolet microscopy is considered especially useful in bringing out differences based on the aromatic amino acid content of proteins, also porphyrin and nucleic acid groups.—*S. H. Hutner.*

MICROTECHNIC IN GENERAL

KIRKPATRICK, J., and LENDRUM, A. C. **Further observations on the use of synthetic resin as a substitute for Canada balsam.** *J. Path. & Bact.*, **53**, 441–3. 1941.

The polystyrene mounting medium previously described (abs. in Stain Techn., **15**, 77, 1940; *cf.* Hutner, Stain Techn., **16**, 177, 1941) is improved by replacing the tricresyl phosphate plasticiser with dibutyl phthalate as follows: mix 5 cc. of dibutyl phthalate with 35 cc. of xylene; dissolve 10 g. of polystyrene ("Distrene 80") in the mixture. The new medium is less tacky at the edge of the coverslip. Preparations with the newer medium have not deteriorated after 14 months. The lapse of time since the earlier publication has afforded further proof that delicate stains, such as Romanowski mixtures, are well preserved. In one uncontrolled series there was fading of hemalum-eosin, which was not observed in other slides so stained. To avoid precipitation of paraffin in the old or new medium, preparations should be mounted from fresh xylene.—*S. H. Hutner.*

DYES AND THEIR BIOLOGICAL USES

HENDERSON, H. J., and LONG, E. R. **Effect of chlorin-e-rhodin-g on experimental tuberculosis.** *Proc. Soc. Exp. Biol. & Med.*, **48**, 435–7. 1941.

Chlorin-e and rhodin-g, substances which are produced when chlorophyll is broken down, together inhibit the growth of tubercle bacilli *in vitro*. They do not affect the course of experimental tuberculosis in rabbits. The pigment accumulates in large quantity in phagocytic cells, in spleen, and in bone marrow. Pigmented cells frequently are concentrated on the border of tubercles.—*M. S. Marshall.*

MOORE, MAURICE L., and MILLER, CHARLES S. **Sulfonamidothiazolones.** *J. Amer. Chem. Soc.*, **63**, 2781. 1941.

The preparation and properties of a number of sulfonamidothiazolones are described. Sixteen 2-sulfanilamido-4-thiazolones have been tested for their chemotherapeutic effect against experimental streptococcal and pneumococcal infections in a standard stock strain of white mice, and show promising activity. The antistreptococcal activity was determined by the oral administration of daily doses of 5 mg. for four days to 20-g. mice experimentally infected with 1000 M.L.D. of a virulent strain of β-hemolytic streptococcus. The initial dose was given at the same time as the intraperitoneal injection of the infective culture. The antipneumoccoccal activity was determined by the oral administration of 20 mg. doses to mice infected with 100 M.L.D. of type I pneumococci at the time of the first dose of compound. Five additional doses were administered at 7, 24, 48, 72 and 96 hr.

In order to correlate the chemotherapeutic effect of these compounds, the relative absorption and blood concentration of 2-sulfanilamido-5-ethyl-4-thiazolone for comparison with sulfanilamide, sulfapyridine and sulfathiazole was determined in mice at a dosage of 0.25 g. and 1.0 g. per Kg. of body weight. It was found that 2-sulfanilamido-5-ethyl-4-thiazole was absorbed as rapidly and its maximum concentration was higher on both levels than for the other three compounds. It also appeared to be completely eliminated from the blood between 8 and 24 hr. after administration at both dose levels.—*A. P. Bradshaw.*

RAIZISS, GEORGE W., and CLEMENCE, LEROY W. **2-Sulfanilyl-aminothiazoline.** *J. Amer. Chem. Soc.*, **63**, 3125. 1941.

The preparation and chemical properties of 2-sulfanilyl-aminothiazoline (sulfathiazoline) and related compounds are described. In tests on various species of animals, sulfathiazoline was found to be of low toxicity. When given by mouth it is absorbed quickly into the blood stream. The therapeutic effect of this compound in mice with types II and III pneumococcus infection was about the same as observed with sulfathiazole. In *Staphylococcus aureus* infection, sulfathiazoline proved to be superior to sulfathiazole.—*A. P. Bradshaw.*

SPRAGUE, J. M., KISSINGER, L. W., and LINCOLN, R. M. **Sulfonamido derivatives of pyrimidines.** *J. Amer. Chem. Soc.*, **63**, 3028. 1941.

The substitution of pyridyl and thiazolyl radicals into the *N* position of sulfanilamide has resulted in compounds of improved therapeutic value. In continuing the study of heterocyclic derivatives of sulfanilamide, a number of sulfonamidopyrimidines, in general, exhibit a high order of activity in experimental infection.—*A. P. Bradshaw.*

WRIST, HAROLD, and JENKINS, GLENN L. **Heterocyclic derivatives related to sulfanilamide. I. The quinoline analog of sulfanilamide and derivatives.** *J. Amer. Chem. Soc.*, **63**, 2943. 1941.

The successful use of sulfanilamide and its derivatives in chemotherapy made it desirable to prepare quinoline analogs of two basic types, namely, 5-aminoquinoline-8-sulfonamide and 8-aminoquinoline-5-sulfonamide. Two other related compounds were prepared: 5-nitro-*N*-(2-pyridyl)-8-quinolinesulfonamide and 5-nitro-*N*-(2-thiazyl)-8-quinoline sulfonamide. The compounds are being tested pharmacologically and bacteriologically.—*A. P. Bradshaw.*

ANIMAL MICROTECHNIC

BRAND, E. **Über eine brauchbare Gegenfärbung zur Holzerschen Darstellungsmethode der faserigen Neuroglia.** *Zts. gesam. Neurol. U. Phychiat.*, **172**, 531–5. 1941.

The author proposes a picro-fuchsin counterstain for the Holzer procedure in staining neuroglia. The technic recommended is:

Prepare 10–15 mμ frozen sections of formalin-fixed nervous tissue and stain by Holzer's method as given in Spielmeyer's *Technik* (1930), but differentiate less than usual. Flush the slide with xylene, blot with paper; absolute alcohol, blot; 95% alcohol, blot. Work rapidly. Apply several drops of the picrofuchsin stain, 10–15 sec., pour off and blot. (The picrofuchsin is made by mixing 7 parts of saturated aqueous picric acid with 5 parts of 1% aqueous acid fuchsin, when used for brain; or 2 parts of the former to 1 part of the latter, when used for spinal cord.) Apply absolute alcohol or better still, isopropyl alcohol, then two applications of xylene, blotting the slide after each. Cover in balsam.

Glia fibers stain blue or blue-black with a violet cast against a contrasting ground. The method gives improved differentiating coloration between fibrillar and cytoplasmic structures especially in pathologic gliosis.—*H. A. Davenport.*

DE LA FUENTA, VICTORINA. **A new method of staining with Wright's solution.** *J. Lab. & Clin. Med.*, **27**, 351–2. 1941.

The following technic is simple, rapid, and when applied to a blood film, reveals the structures clearly. Cover the air-dried smear with a few drops of Wright's

stain. Blow on the smear with the breath until it becomes pinkish-violet in color (about 10 sec.) Wash with tap water. With tap water running slowly over the slightly tilted slide, pour on a few drops of Wright's stain quickly. Wash, dry and examine with the oil immersion lens. Blowing the smear accelerates staining from minutes to seconds. Washing with the stain itself dissolves any precipitated particles of stain.—*John T. Myers.*

HEATH, JAMES P. The nervous system of the kelp crab, Pugettia producta. *J. Morph.*, **69**, 481–500. 1941.

Methylene blue failed to differentiate the nervous system well in this species. Of the several stains used, toluidine blue proved to be most satisfactory, since it usually gave a strong coloration to the nerve sheaths.

The author did not indicate the sources of the dyes, their certification numbers, concentration of solutions, or methods of application.—*Elbert C. Cole.*

FRENCH, C. A combined stain for fat and elastic tissue. *Arch. Path.*, **30**, 1243. 1940.

For simultaneous staining of fat and elastic fibers, the author recommends the following method of embedding: Fix tissues in a solution of formaldehyde (strength not given) for 24 hr. or longer. Wash in running water for 5 hr. Place in 5% aqueous solution of gelatin (Knox) at 37° C. for 12–24 hr.; in 10% gelatin at 37° C. for 6–12 hr. Embed in 10% gelatin. Harden in refrigerator for 2–4 hr. Before cutting, place individual blocks in formaldehyde solution for at least 12–24 hr. Cut sections on freezing microtome at 10 μ. Before staining, place sections in formaldehyde for 12–24 hr.

The staining procedure recommended is: Rinse section well in distilled water. Dip several times in 70% alcohol. Place for 15 min. in the following fat and elastic tissue stain, warmed to not more than 45° C.: Weigert's elastic tissue stain (ripened), 10 ml.; distilled water, 2 ml.; saturated solution of "scarlet red" (presumably Sudan IV) in equal parts of acetone and 70% alcohol, 8 ml. Rinse in 70% alcohol; then in distilled water. Place in Delafield's (or other) hematoxylin for 1–2 min. Rinse in distilled water. Differentiate in 0.5% acid alcohol, if desired. Immerse in weak ammonia until the section becomes blue. Rinse in distilled water. Transfer to a slide, drain, and mount in glythrogel. (*From J. Lab. and Clin. Med.*, **26**, 1991–2.)

KAY, W. W., and WHITEHEAD, RAYMOND. The role of impurities and mixtures of isomers in the staining of fat by commercial Sudans. *J. Path. & Bact.*, **53**, 279–84. 1941.

A study of many dyes of the Sudan III and IV classes indicated that mixtures of purified Sudans stained more strongly than the individual components, and that commercial impure Sudans stained better than the pure Sudans. Sudan IV (British Drug Houses No. 555722) was the most satisfactory.—*S. H. Hutner.*

KRAJIAN, A. A rapid method of staining fat in frozen sections with osmic acid. *Arch. Path.*, **30**, 766. 1940.

The following procedure is recommended for staining fat: Fix tissue in 10% formaldehyde for 24 hr. Cut frozen sections 10 μ thick. Bring to a boil a 1% aqueous osmic acid in a Pyrex test tube and pour into a small Stender dish. Place the section in the hot osmic acid solution and keep in a paraffin oven at 60° C. for 5 min. Wash in a basin of tap water. Counterstain in 1% aqueous eosin or phloxine for 1 min. Wash rapidly in tap water. Transfer to a slide and allow to drain. Mount in 3 large drops of glycerin jelly (previously melted in paraffin oven or water bath). Results: The fat globules are black or gray black; the background is red.

For emergency examination, bring a 10% solution of formaldehyde to boil in a Pyrex test tube (60 ml. capacity); drop a thin piece of biopsy or autopsy material into it, and place the specimen in an oven at 60–65° C. for 10 min. Cut thin frozen sections and stain by the method described.

It is not necessary to discard the used osmic acid solution. It may be poured back into the stock bottle and used over and over.—(*From J. Lab. and Clin. Med.*, **26**, 1991.)

PEERS, J. H. **A Modification of Mallory's phosphotungstic-acid-hematoxylin stain for formaldehyde-fixed tissues.** *Arch. Path.*, 32, 446. 1941.

After formaldehyde fixation the author recommends the following schedule: Deparaffinize sections and bring down to water. Mordant 3 hr. in a saturated solution of $HgCl_2$ in a paraffin oven at 57° C. Rinse briefly. (The $HgCl_2$ solution may be used repeatedly.) Place in "compound solution of iodine" (i.e. Lugol's solution) for 5 min. Rinse. Place in 5% aqueous $Na_2S_2O_3$ for 5 min. Rinse. Place in 0.25% $KMnO_4$ for 5 min. Rinse. Place in 5% aqueous oxalic acid for 5 min. Wash well. Stain overnight in phosphotungstic acid hematoxylin. Wash briefly in tap water, dehydrate in alcohol or acetone, clear in xylene and mount in balsam.

The technic gives results almost identical with those of the original method as applied to tissue fixed in Zenker's solution. Neuroglia, fibroglia, and myoglia fibrils, belpharoplasts, nuclei, red blood cells, and fibrin are deep blue, while collagen is brownish red. (*From J. Lab. & Clin. Med.*, 27, 701.)

SAZERAC, R., and POUZERGUES, J. **Recherche du bismuth dans les cellules et les tissus animaux. Formation de cristaux caractéristiques.** *Ann. Inst. Pasteur*, 66, 90–5. 1941.

To demonstrate phagocytized bismuth in smears or sections, the following fresh aqueous mixture is prepared: 2% *o*-hydroxyquinoline, 1 cc.; 4% KI, 1 cc.; 3% HNO_3, 8 cc. The mixture forms an orange-red double salt with bismuth up to a dilution of 1:1,000,000. After intraperitoneal injection of an aqueous suspension of bismuth, proceed as follows: Smear the peritoneal fluid, dry, and fix with heat. Add 1 drop of fresh reagent, then a coverslip. The phagocytes are colored yellow, grains of bismuth orange-red, and the crystals grow from these granules to resemble the sheaves of prisms of certain osazones. Section tissues as usual after formalin fixation, and add the reagent after removal of paraffin. Crystallization is favored by heating the slide at 80° for 2–3 min.—*S. H. Hutner.*

ZAHL, P. A., and WATERS, L. L. **Localization of colloidal dyes in animal tumors.** *Proc. Soc. Exp. Biol. & Med.*, 48, 304–10. 1941.

Evans blue, lithium carmine and nine other dyes were injected intravenously into tumor-bearing mice. Tumors were studied histologically. Acid dyes did not localize in the tumor cells but in the surrounding stroma. Further localization took place within the tumor at the interface between viable and necrotic tissue. Small repeated doses did not localize in greater amounts than a single injection given in the same amount. There was a difference in behavior between colloidal dyes and colloidal carbon.—*M. S. Marshall.*

PLANT MICROTECHNIC

BALL, ERNEST. **Microtechnique for the shoot apex.** *Amer. J. Bot.*, 28, 233–43. 1941.

The author describes a series of improved methods, from fixation to sectioning, for preparing sections of shoot apices of vascular plants. Chamberlain's formula (5 cc. glacial acetic acid; 5 cc. commercial formalin, and sufficient 70% ethyl alcohol to make 100 cc. of solution) is modified by replacing the ethyl alcohol with the same volume of methyl, iso-propyl, *n*-propyl or tri-butyl alcohol, and the acetic acid with an equivalent amount of another fatty acid, e.g. 3.7 cc. formic (88% sol.), 6.5 cc. propionic, 8 cc. butyric, or 9.4 cc. valeric. Similar modifications of Juel's solution and of several aqueous fixatives are also tried. Fixation is for 24 hr., followed by washing in a 70% solution of the corresponding alcohol. A method for dehydrating tissues in alcohol over CaO and $CuSO_4$ is also described.

Only ethyl alcohol was found to give good results when used in the fixing solutions. Acetic acid was found to be the most suitable of the fatty acids in preparing fixatives. Several phases of the effects of different fluids used in the preparation of sections of shoot apices for microscopic examination are discussed.—*T. M. McMillion.*

MICROÖRGANISMS

AMBROSIONI, P. **Modificazione del metodo di Gins per la colorazione del B. difterico.** *Ann. Igiene*, **50**, 228–30. 1940.

The author's modification of the Gins method is simple and is claimed to be more efficient than the original. The procedure: After fixing smear in ordinary fashion, let the first stain (1 g. crystal violet, 15 cc. glacial acetic acid, 285 cc. distilled water) act for 10–15 sec. Wash. Apply second stain (chrysoidin, 2 g. in 100 cc. distilled water) for 20–30 sec. Wash. Mordant (Lugol's solution 99 cc., lactic acid 1 cc.) 5 sec. Wash. Dry. Examine.—*Carl Lamanna.*

FLEMING, ALEXANDER. **Some uses of nigrosin in bacteriology.** *J. Path. & Bact.*, **53**, 293–6. 1941.

The following solution is recommended for negative staining of bacteria: nigrosin, water-soluble (Gurr), 10 g.; water, 90 cc.; formalin, 10 cc. The nigrosin may be mixed with the bacteria and spread either like a blood smear or with a loop. Stained bacteria can be overspread with nigrosin to demonstrate capsules in the following manner: As nigrosin solution is a decolorizer, dry slides rapidly. To combine spore-staining and negative staining, stain spores the usual way with carbol fuchsin, rinse off excess dye with water and alcohol. Cover with the nigrosin solution 5 min. or longer, wash off with tap water. Place a drop of nigrosin on one end of the slide, spread with another slide over the stained portion and allow to dry. Bright red spores show against the unstained bodies. (This latter is a slight modification of the method described by Dorner in 1922, but no credit is given.)—*S. H. Hutner.*

MANOUÉLIAN, Y. **Variations dans l'argyrophilie des spirochètes.** *Ann. Inst. Pasteur*, **66**, 83–9. 1941.

Faintly stained or unstained spirochetes are not uncommon in tissues known to be infectious. Tissues kept in formalin for 10–15 years may be impregnated perfectly; yet sometimes impregnation, for unknown reasons, may fail both in fresh and old tissues. Absence of morphologically demonstrable spirochetes in infectious tissues should therefore not be ascribed to a filterable stage in the life history of the spirochete.—*S. H. Hutner.*

PIJPER, ADRIANUS. **Dark-ground studies of Vi agglutination of B. typhosus.** *J. Path. & Bact.*, **53**, 431–6. 1941.

This continuation of the author's studies on bacterial agglutination (abs. in Stain Techn., **14**, 73, 1939) is illustrated with remarkable photomicrographs of H, O and Vi agglutination, which clearly show the role of flagella and of the remainder of the cell in these morphologically distinct types of agglutination. Three 16-mm. motion picture films have been prepared by the author illustrating (1) sunlight dark-field technic and motility of typhoid bacteria, (2) O aggutination and (3) H agglutination of typhoid bacilli. A film on Vi agglutination is in preparation.—*S. H. Hutner.*

PRYCE, D. M. **Sputum film cultures of tubercle bacilli: a method for the early observation of growth.** *J. Path. & Bact.*, **53**, 327–34. 1941.

Micro-culture of tubercle bacilli offers a possibility of more rapid diagnosis. Other microbes on a sputum smear are destroyed by acid, the smear is then incubated in contact with a clear culture medium, and the microcolonies of tubercle bacilli are stained so as to be visible with low powers of the microscope. Two methods are described in detail: (1) Spread sputum on the flat or slightly concave bottom of a 4 in. Petri dish. Treat the dried film with 15% H_2SO_4 for 5 min., pipette off the acid, flood the smear with 5 cc. of distilled water and allow to act for about 1 min. Repeat the washing 1–2 times. Add the medium (prepared by laking citrated human blood with an equal volume of 1% saponin) and incubate 1 week. Wash off the medium with water and stain with Ziehl-Neelsen as usual. Counterstaining of the colonies is unnecessary. (2) Smear and dry one or more circular patches of sputum on a glass slide. Immerse in melted vaseline a bakelite, hard fiber, or glass ring about 1–2.5 cm. diameter. Drain off excess vaseline and apply the ring about a patch of sputum. Apply acid and rinse as before, then incubate in contact with the medium either in a moist

chamber made from a Petri dish or on a slide closed with a vaselined coverslip. Stain as before.

Notes: Rings may be cut from tubing, then sandpapered. Vaseline does not interfere with staining if the stain is rewarmed before rinsing. All solutions used must be sterile. If a closed chamber is used for incubation, the blood should be well oxygenated and adequate in amount. Besides killing non-acid-fast organisms, the acid coagulates and "fixes" the mucus; thick layers of mucus may be evaporated. Laryngeal swabbings give better results than sputa. The technic can be applied to pus and caseous material.—*S. H. Hutner.*

SHERIDAN, B. W. **A rapid method of dehydrating and clearing iron-hematoxylin-stained fecal smears.** *J. Lab. & Clin. Med.*, **27**, 254. 1941.

The following method shortens the preparation of wet-fixed stained smears for the identification of intestinal protozoa. Fix in Schaudin's solution (with glacial acetic acid added up to 5%) for 5 min. Place serially in: tap water, 3 min.; 95% alcohol (with iodine added to make a port wine color), 3 min.; 4% $FeNH_4(SO_4)_2$, 15 min.; rinse in tap water; apply 0.5% aqueous hematoxylin, 10 min.; 0.25% $FeNH_4(SO_4)_2$, 10 min.; running water, 1 min.; cellosolve (ethylene glycol monoethyl ether), 10 min.; mount in balsam. This method is quick, satisfactory and as permanent as any other technic.—*John T. Myers.*

HISTOCHEMISTRY

HAMAZAKI, Y. **Über den Chemismus des Modellversuches für die Karbolfuchsin-Jod-Methode (eine neue Nachweismethode für Nucleinsaure und Purinderivate).** *Trans. Soc. Path. Jap.*, **30**, 111–5. 1940.

After fixation in chromic acid solutions, animal (but not plant) nucleic acids are stained by a procedure which appears to combine features of the Fuelgen and Gram technics. Details of the staining procedure are not given, but the staining solution appears to consist of a mixture of Lugol's iodine and basic fuchsin. Staining is followed by decolorization with a strongly acid solution of $NaHSO_3$. The difference between plant and animal nucleic acids is ascribed to the presence of desoxyribose in animal nucleic acid and ribose in plant nucleic acid. Oxidation of the desoxyribose by the CrO_3 creates a keto-enol group with which the iodine and fuchsin combine, and which resists decolorization. Oxidation of ribose does not create the necessary keto-enol group. This theory is based on the formation of a similar purple dye complex from desoxycholic acid but not from cholic acid. Treatment of cholic acid with CrO_3 renders it stainable. Since the procedure is considered a test for keto-enol groups, it is pointed out that products of nucleic acid hydrolysis, such as adenine and guanine, which on CrO_3 oxidation yield products containing the keto-enol group, also stain purple.—*S. H. Hutner.*

KNAYSI, GEORGES. **On the use of basic dyes for the demonstration of the hydrolysis of fat.** *J. Bact.*, **42**, 587–9. 1941.

Several basic dyes other than Nile blue can be used to demonstrate the hydrolysis of fats by microörganisms if the dyes are converted to their free bases, by the following procedure: Liberate the free base with *N* NaOH added drop by drop until precipitation is complete; filter; wash the precipitate with distilled water adjusted to about pH 7.5; dry and store the base; or use it to saturate the fat incorporated with the culture medium, which is then sterilized by autoclaving. Nile blue, neutral red, methylene blue, and malachite green have been tested; the colors of the neutral fat are red, orange-yellow, red, and olive green respectively; the corresponding colors of the free fatty acids are blue, red, blue and blue-green. Any basic dye with the color of the free base differing from that of the salt could be used. The medium in which the colored fats are used should be neutral or slightly alkaline, highly buffered, and should not contain fermentable sugars because acids in the aqueous phase extract the dye from the fat forming a dye salt in the medium and leaving the fat drops uncolored.

Nile blue is often toxic, but this may be overcome by using it as follows: Shake 1 ml. of a saturated aqueous solution of its sulfate or chloride with 10 ml. neutral fat, separate the red fat from the blue solution in a separatory funnel, wash with distilled water, and sterilize by autoclaving. One ml. of this fat shaken with 100 ml. of agar medium is used for plating.—*Virgene Kavanagh.*